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REMARKS

Claims 1-211 were pending prior to this response, with claims 47-211 being withdrawn due to a restriction requirement. By the present communication, new claims 212-216 have been added, claims 25, 26, 28, 33 and 47-211 have been cancelled without prejudice, and claims 1, 11-14, 20, 21, 27, 29, 31, 32, 34, 35 and 37 have been amended to define Applicants' invention with greater particularity. Accordingly, claims 1-24, 27, 29-32, 34-46 and 212-216 are currently pending.

The Objection to the Claims

Claims 20, 21 and 29 have been objected to due to alleged informalities. With regard to claims 20 and 21, the Office Action indicates the presence of an extraneous dash preceding the claim number. To overcome the objection, claims 20 and 21 have been amended to delete the extraneous dash by reciting, respectively, "The method of claim 1" and "The method of claim 2". With regard to claim 29, the Examiner points out misspelling of the word "biomolecule". Claim 29 has been amended to delete the word "biomolecule", thus rendering the rejection moot as to claim 29. In view of the amendments to claims 20, 21 and 29, Applicants respectfully request reconsideration and withdrawal of the objection to the claims for including informalities.

The Objection the Specification

Applicants respectfully traverse the objection to the specification as allegedly failing to provide: "antecedent basis for the concept of labeling the nucleic acid prove with a biotinylated substrate wherein the biotinylated substrate comprises a core fluorophores, a spacer connected to the fluorophores by a first connector and a connector to a bioactivity or biomolecule of interest and 2 functional groups or wherein the biotinylated substrate comprises a core fluorophores, a spacer connected to the fluorophores by a connector and connected to a bioactivity or biomolecule of interest by a second connector, and a quencher component attached to a fluorophores by a polymer" (Office Action, page 2). The Examiner acknowledges that the

specification discloses microdroplets labeled with such biotinylated substrates but allegedly does not describe attaching such substrates to nucleic acid probes for hybridization and detection purposes. To overcome the alleged lack of antecedent basis, new independent 212 has been added, which recites that the polynucleotides are contained within clones which are encapsulated for expression within microdroplets that have a biotinylated substrate attached to the microdroplet via a biotin-avidin-biotin bridge. The large substrates including a core fluorophore are required to fluoresce upon contact with a specific enzyme. Former claims 25, 26 and 28 have been cancelled and remaining claims 27, 29-32, and 34-40 are now dependent upon, and thus incorporate, all limitations of new claim 212, which requires that the biotinylated substrate is attached to a microdroplet that encapsulates the plurality of polynucleotides through a biotin-streptavidin-biotin bridge and thus, allows isolation of a microdroplet using the flow cytometer based on fluorescence resulting from the presence of an enzyme specific for the substrate. Applicants submit that the Specification's description of GMD-attachable fluorescence resonance energy transfer substrates and fluorogenic substrates, as acknowledged by the Examiner, is sufficient to establish antecedent basis for the subject matter of new independent claim 212 and claims dependent thereon. Therefore, Applicants submit that the grounds of the objection have been overcome and respectfully request reconsideration and withdrawal of the objection to the Specification.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claims 28-33 and 35-40 under 35 U.S.C. § 112, first paragraph, as lacking enablement because the Specification allegedly lacks an enabling description of methods for identifying a polynucleotide by contacting a plurality of polynucleotides with a nucleic acid probe labeled with a biotinylated substrate (Office Action, page 3). Claims 28 and 33 have been cancelled without prejudice and the remaining claims at issue have been made dependent upon new claim 212. Therefore, Applicants will address the rejection with regard to new claim 212 and claims dependent thereon.

Applicants submit that claims dependent upon new claim 212 that recite a biotinylated substrate having core fluorophore decorated with connectors spacers quenchers, functional groups and/or a polymer are enabled by the Specification's description of GMD-attachable fluorescence resonance energy transfer substrates and fluorogenic substrates at pages 120-124, particularly the following, which describes formation of the "bridge" that attaches the substrate to the microdroplet, in this case an agarose microdroplet:

> The GMDs are made with agarose derivatized with biotin, which is commercially available (One Cell Systems). After appropriate colony growth, streptavidin is added to serve as a bridge between a biotinylated substrate and the biotin-labeled agarose. Finally, the biotinylated substrate will be added to the GMD and captured within the GMD through the biotin-streptavidin-biotin bridge. The bacterial cells will be lysed and the enzyme released from the cells. The enzyme will catalyze the hydrolysis of the substrate, thereby increasing the fluorescence of the substrate within the GMD. The fluorescent substrate will be retained within GMD through the biotin-streptavidin-biotin bridge and thus, will allow isolation of the GMD based on fluorescence using the flow cytometer. The entire microdrop will be sorted and the DNA from the bacterial colony recovered using PCR techniques. This technique can be applied to the discovery of any enzyme that hydrolyzes a substrate with the result of an increased fluorescence. Examples include but are not limited to glycosidases, proteases, lipases, ferullic acid esterases, secondary amidases, and the like. (Specification, page 120)

In addition to this general description, Applicants provide a specific example of chemical synthesis of biotinylated substrate that can be used to detect esterases and two schematic drawings of substrates useful for other types of enzymes.

In view of the above disclosure, Applicants respectfully submit that those of skill in the art would know how to make and use the invention methods, as recited by new claims 212 and claims dependent thereon, without undue experimentation. Accordingly, reconsideration and withdrawal of the rejection for alleged lack of enablement are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 1-46 under 35 U.S.C. § 112, second paragraph, as being indefinite due to an alleged lack of clarity regarding the relationship between the individual method steps. In particular, the Examiner is concerned that claim 1 fails to require a wash step in which unbound probes are removed. Applicants disagree that those of skill in the art would be confused by omission of such a requirement due to the overall logic of the amended claims and the full disclosure of the Specification. In the invention methods as defined by claim 1, the analyzer detects an event that is generated or changed by hybridization of the probe to a naturally occurring polynucleotide of interest. There is no need to remove unbound probe since failure of the probe to hybridize does not result in the phenomenon that is detected. Detection can take place in liquid, for example, within the capillaries of a capillary array or the wells of a microtiter plate. Detection of a hybridization event and analyzers that can be used in practice of the invention include, for example, those wherein hybridization to polynucleotide generates or causes a change in a fluorescent signal and the analyzer is a flow cytometer, or causes a modulation in magnetic field and the sensing device is a Super Conducting Quantum Interference Device, as required in claim 17, or those wherein the analyzer is a multipole coupling spectroscopy device and the hybridization generates a change in spectroscopic signal, as required in claim 18, as is known to those of skill in the art (See U. S. Patent No. 6,338,968 and 6,340,568 of record herein). Thus, Applicants submit that insertion of a method step requiring removal of unbound probe would introduce an unnecessarily limitation into the claims.

In addition, the Examiner asserts that there is lack of clarity as to whether there is intended to be a distinction between the polynucleotide and the "polynucleotide of interest" in claim 1. To address this potential confusion, claim 1 has further been amended to recite that the "nucleic acid probe comprises a sequence complementary to a sequence that produces a bioactivity or biomolecule of interest" and that a "polynucleotide of interest" is one among the "plurality of polynucleotides" in the assay to which such a probe hybridizes. The polynucleotide of interest is then identified by detecting a polynucleotide to which the probe has hybridized.

The Examiner also asserts that it is unclear whether the phrase "in liquid phase" in the preamble to claim 1 is intended to further limit the claims (Office Action page 6) because it is unclear, for example how the interior of a microenvironment can be considered "in liquid phase." To overcome this alleged lack of clarity, claim 1 has been amended to delete the phrase "in liquid phase" from the preamble. The specification makes abundantly clear that the contacting, hybridization, and detecting, as defined by amended claim 1, can take place "in a liquid phase," for example in a capillary tube or well of a microtiter plate, but the method is not required to take place in liquid phase. In addition, embodiments of the invention employing encapsulation in a microdroplet are defined by new claim 212, which omits any requirement that the incubating of the clones encapsulated in the microenvironment and the detecting takes place "in liquid phase."

With regard to the Examiner's assertion that claim 14 lacks clarity in the use of the phrase "the dielectric signature of the clone", claim 14 has been amended to delete reference to a "clone", thus rendering the basis of the rejection moot.

With regard to the Examiner's assertion that claim 25 lacks clarity in the use of the phrase "further comprising encapsulation of the polynucleotide in a microenvironment", claim 25 has been cancelled, thus rendering the basis of the rejection moot.

With regard to claims 29-33 and 35-40, the Examiner asserts that the recitation of "functional groups" is a source of indefiniteness due to failure to state the function of the groups. To further define the meaning of the phrase at issue, claim 29 has been amended to add the phrase, "wherein each functional group determines specificity of the substrate for an enzyme of interest." The Specification teaches that such functional groups can be selected from "straight and branched alkanes, mono- and oligosaccharides, unsaturated hydrocarbons and aromatic groups" (Specification, page 123). Applicants submit that the amendment to claim 29 resolves any possible lack of definiteness regarding meaning of the term "functional groups" in claim 29 and those claims dependent thereon.

With regard to alleged lack of definiteness of the phrase "the bioactivity or biomolecule of interest" as used in claims 29-33 and 35-40, the phrase has been deleted from claims 29 and 35, thus rendering moot the grounds for the rejection.

With regard to the assertion that the phrase "selected from the groups consisting of" in claim 32 is improper Markush language because the claim does not recite groups, but rather recites function moieties, Applicants have amended claim 32 to recite "selected from the group of functional moieties consisting of." Applicants submit that the amendments to claim 32 utilize proper Markush language, thus obviating the grounds for the rejection.

With regard to the alleged lack of antecedent basis for the term "the clurophore" in claim 35, Applicants have amended claim 35 to correct an obvious typographical error by replacing the phrase at issue with "the fluorophore" for which antecedent basis is provided in line 2 of claim 35, among others, thus overcoming the grounds of the rejection.

With regard to alleged indefiniteness of the phrase "encodes a small molecule" in claim 43, the Examiner alleges that the phrase "small molecule" does not have a defined meaning in the art and the term "small" is not defined in the specification. However, Applicants submit that the phrase "small molecule" as used in Applicants' specification and claims does not refer specifically to the size of the molecule. Those of skill in the art would understand that, as used in claim 19, the phrase "small molecule" is used to distinguish a chemical molecule or complex, such as a non-proteinaceous enzyme, from molecules containing amino acids or nucleic acids, either of which may be smaller in terms of molecular weight than a large chemical complex. Since the definiteness of claim language is determined with reference to the understanding of those of skill in the art, and Applicants submit that those of skill in the art would readily understand the meaning of the phrase "small molecule" as used in claim 43, Applicants submit that claim 43 is definite as written.

In view of the amendments and above remarks, Applicants respectfully submit that all claims meet the requirements for definiteness under 35 U.S.C. § 112, second paragraph and reconsideration and withdrawal of the rejection are respectfully requested.

The Double Patenting Rejection

Applicants respectfully traverse the rejection of claims 1-28, 34 and 41-46 for nonstatutory double patenting over the claims of U.S. Patent No. 6,174,673 and the provisional nonstatutory double patenting rejection over the claims of U.S. Patent Application Nos. 09/738,871 and 09/685,432.

Applicants submit herewith a Terminal Disclaimer disclaiming the terminal part of any patent granted on the subject matter of the above-identified Application Serial No. 09/975,036, that would extend beyond the expiration date of U.S. Patent No. 6,174,673 as well as any patent that may be granted on U.S. Application Serial Nos. 09/738,871, filed December 14, 2000, and 09/685,432, filed October 10, 2000, the terminal disclaimer to be effective only so long as U.S. Patent No. 6,174,673 and any patent that may be granted on the above-identified Application Serial Nos. 09/975,036, 09/738,871, and 09/685,432, are co-owned.

In view of the Terminal Disclaimer submitted herewith, Applicants submit that the grounds of the rejection for non-statutory double patenting are overcome. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection Under 35 U.S.C. § 102(e)

Applicants respectfully traverse the rejection of claims 1-5, 15, 16, 19-27, and 41-46 as allegedly being anticipated under 35 U.S.C. § 102(e) by Thompson et al. (U.S. Patent No. 5,824,485; hereinafter "Thompson"). Applicants submit that the invention methods for identifying a *naturally occurring* polynucleotide of interest, as defined by amended claim 1, distinguish over the disclosure of Thompson by requiring: "contacting a plurality of naturally occurring polynucleotides derived from at least one organism with at least one nucleic acid probe that comprise a sequence complementary to a bioactivity or biomolecule of interest under conditions that allow hybridization of the probe to polynucleotides having sequences of interest; and identifying a naturally occurring polynucleotide of interest with an analyzer that detects a polynucleotide to which a probe has hybridized."

Applicants submit that Thompson is silent regarding methods for detecting a *naturally occurring* polynucleotide of interest by detecting hybridization events.

With regard to the subject matter of new claim 212 and claims dependent thereon, Applicant submits that Thompson fails to disclose screening of a clone comprising a polynucleotide encapsulated within a microdroplet having a substrate attached to the microdroplet via a biotin-avidin-biotin bridge for any purpose.

Instead, Thompson's methods, as acknowledged by the Examiner in the Office Action, are concerned with "combinatorial gene expression libraries" that may be prescreened with "DNA representing non-primary metabolism related genes . . .labeled using random priming, and used as a probe to pre-screen the library" (Office Action, page 11). Combinatorial gene expression libraries do not contain naturally occurring polynucleotides, since by definition a combinatorial gene is one that has been altered or pieced together by methods disclosed by Thompson. For example, to reduce the number of clones that need to be screened, Thompson describes pre-selection of DNA fragments for the screening library using probes and refers to this process as "biasing" a library. Such probes are described as being "prepared from known genes that may be related to or are involved in producing compounds of interest" (Thompson, Col 32, lines 6-7). However, rather than using the probes for screening (e.g., identifying naturally occurring molecules having a nucleotide sequence complementary to the probes) of a library of naturally occurring molecules, as in Applicants' claim 1, Thompson uses the activity probe concept for preparing "chimeric" and "biased" *combinatorial expression libraries*" (See Thompson, Section 5.1.6) prior to screening.

Applicants provide extrinsic evidence in support of the meaning of "combinatorial" as used in Thompson to distinguish such teaching from Applicants' teaching and claims directed to screening of naturally occurring molecules. Exhibit A is a print out from an internet site that includes a description of Neugenesis' combinatorial biology technology, which creates "combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology." (http://www.neugenesis.com/) Clearly,

Applicants' claims are not directed to combinatorial approaches to identifying enzyme activities encoded by naturally occurring gene clusters, since Applicants are not manipulating the DNA to generate variants.

Exhibit B is a printout from the internet site of the Koide Group, from University of Pittsburgh (http://www.pitt.edu/~sparano/group/). As you will note, the study of Natural Products is separate and distinct from the study of Combinatorial Libraries. Exhibit C provides a glossary of terms used in Medicinal Chemistry. On page 4, the term combinatorial synthesis is described as "...combining sets of building blocks" e.g., ligating together individual genes of a gene cluster.

Thus, in view of Thompson's use of probes for screening in the context of preparing combinatorial libraries, Applicants submit that Thompson fails to disclose use of detectably labeled probes for screening of such libraries to detect *naturally occurring polynucleotides of interest*. Accordingly, Applicants respectfully submit that Thompson fails to disclose each and every element of independent claims 1 and 212 (and claims dependent thereon) as would be required to establish anticipation under 35 U.S.C. 102(e).

The Rejection under 35 U.S.C. § 103(a)

A. Applicants respectfully traverse the rejection of claims 1-11, 14-16, 19-28, 34 and 41-46 under 35 U.S.C. 103(a) as allegedly being unpatentable over Thompson in view of Blumenfeld (U.S. Patent No. 6,228,580; hereinafter "Blumenfeld"). Applicants' remarks above regarding the deficiencies of Thompson for disclosing the invention methods apply equally and are incorporated here. In addition, Applicants submit that Thompson fails to suggest the invention methods because Thompson's focus in using hybridizing probes is to reduce the number of clones in a library by eliminating DNA that encodes genes related to primary organism functions in a gene subtraction method and biotinylated molecules are used in assembly of a combinatorial chimeric pathway expression library (See Thompson Section 5.4.6. ASSEMBLY OF A COMBINATORIAL CHIMERIC PATHWAY EXPRESSION LIBRARY). There is no

suggestion in Thompson to use a probe (e.g., a labeled probe) to identify a naturally occurring polynucleotide of interest.

The Examiner relies upon Blumenfeld as disclosing the use of nucleic acid hybridization probes with fluorescent moieties in combination with Thompson' use of biotin labeled nucleic acid that can be detected using streptavidin. However, there is no suggestion in the combined disclosure of Blumenfeld and Thompson to attach a enzyme substrate to a microdroplet via a biotin-streptavidin-biotin bridge, as is required in Applicants' new claim 212.

Moreover, Applicants disagree with the Examiner's assertion that "a nucleotide labeled with biotin is considered to be a 'biotinylated substrate'" (Office Action, page 14). The molecules to which Thompson attaches biotin are pieced together to make recombinant molecules upon which probes are used. By contrast, the term "biotinylated substrate" as used in Applicants' Specification and new claim 212 is a molecule upon which an enzyme performs its enzymatic activity and does not "hybridize" to the polynucleotide of interest as does a "nucleic acid probe.". Therefore, Applicants submit that the combined disclosures of Thompson and Blumenfeld do not suggest and would not motivate those of skill in the art to arrive at Applicants' methods of using hybridizing nucleic acid probes, as defined by amended claim 1, or "biotinylated substrates", as defined by new claim 212. Therefore, Applicants respectfully submit that prima facie obviousness of any pending claims is not established over the combined disclosures of Thompson and Blumenfeld. Consequently, reconsideration and withdrawal of the rejection over Thompson in view of Blumenfeld under 35 U.S.C. § 103(a) are respectfully requested.

B. Applicants respectfully traverse the rejection of claims 1-10, 13, 14, 16, 18-27 and 41-46 under 35 U.S.C. 103(a) as allegedly being unpatentable over Thompson in view of Hefti (U.S. Patent No. 6,340,568; hereinafter "Hefti"). Applicants remarks above regarding the deficiencies of Thompson for disclosing or suggesting the invention methods apply equally and are incorporated here.

The Examiner relies upon Hefti to cure the deficiencies of Thompson described above, particularly with respect to use of nucleic acid probes "up to 10,000 nucleotides in length in the method of Thompson" and detection of "molecular binding events" without the need for labeling the probe using multipole coupling spectroscopy. However, Hefti does not overcome the deficiencies of Thompson described above for suggesting to or motivating those of skill in art to arrive at the invention methods for screening a library of naturally occurring polynucleotides, such as one obtained from an environmental source under extreme conditions, to identifying a naturally occurring polynucleotide of interest such as one encoding a novel enzyme, as defined by amended claim 1 and claims dependent thereon. Hefti's focus is in determining the degree of hybridization or near hybridization using the extremely technical methods of MCS. Hefti is not concerned with detecting polynucleotides that encode a bioactivity or biomolecule of interest. In addition, Hefti neither teaches or suggests the invention methods for using an avidin-biotin-avidin bridge to anchor a substrate into a microdroplet for detection of an enzyme encoded by a naturally occurring polynucleotide expressed within the microdroplet, as defined by new claim 212 and claims dependent thereon.

C. Applicants respectfully traverse the rejection of claims 1-17, 19-28, 34 and 41-46 under 35 U.S.C. 103(a) as allegedly being unpatentable over Thompson in view of Hefti and Baselt (U.S. Patent No. 5,981,297). Applicants' remarks above regarding the deficiencies of the combined disclosures of Thompson and Hefti for disclosing or suggesting the invention methods under 35 U.S.C. § 103(a) apply equally and are incorporated here.

The Examiner relies upon Baselt to cure the deficiencies of the combined disclosures of Thompson and Hefti described above, particularly with respect to use of "labeling nucleic acid probes with magnetic molecules and detecting hybridization of probes to polynucleotides of interest using SQUID" (Office Action, page 16). However, Applicants submit that Baselt does not overcome the deficiencies of Thompson and Hefti described above for suggesting to, or motivating those of skill in art to arrive at, the invention methods for screening a library of naturally occurring polynucleotides to identifying a naturally occurring polynucleotide of

interest, as defined by amended claim 1 and claims dependent thereon. Baselt's focus is to use a magnetic field detector, such as SQUID, to determine the presence of multiple analytes, or the concentration of an analyte in a liquid or gas phase and utilizes, with binding molecules (e.g., probes) bound to the sensors. Baselt is not concerned with identifying polynucleotides that encode or may encode an enzyme or other "bioactivity or biomolecule of interest", as the terms are used in Applicants' Specification and claims. In addition, Baselt neither teaches or suggests the invention methods for using an avidin-biotin-avidin bridge to anchor a substrate into a microdroplet for detection of an enzyme produced by a novel source, e.g., encoded by a naturally occurring polynucleotide expressed within the microdroplet, as defined by new claim 212 and claims dependent thereon.

Therefore, Applicants submit that the combined disclosures of Thompson, Hefti and Baselt do not suggest, and would not motivate, those of skill in the art to arrive at Applicants' methods of using hybridizing nucleic acid probes, as defined by amended claim 1, or "biotinylated substrates", as defined by new claim 212 for discovery of naturally occurring polynucleotides of interest". Accordingly, Applicants submit that prima facie obviousness of any pending claims is not established under 35 U.S.C. § 103(a) over the combined disclosures of Thompson, Hefti, and Baselt, and reconsideration and withdrawal of the rejection are respectfully requested.

Conclusion

In view of the Terminal Disclaimer as well as the amendments and remarks above, Applicants believe that all objection and rejections have been overcome and passage of the claims to allowance is respectfully requested.

If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: May 6, 2004

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Enclosures: Exhibit A
Exhibit B
Exhibit C
Terminal Disclaimer

Neugenesis
A Biotechnology Company

COMBINATORIAL BIOLOGY TECHNOLOGY

Neugenesis' combinatorial biology technology (the CombiKARYON™ system) mimics the immune system's ability to generate diversity in antibodies, and expands the application to all heteromeric proteins. The company uses this technology to discover and improve complex proteins. It is an efficient and cost-effective method to improve a protein's stability, affinity, receptor binding capacity, and therapeutic efficacy, thereby enhancing the value of the protein, and decreasing the risk of clinical failure.

Using Combinatorial Biology to Generate Diversity

An outgrowth of Neugenesis' protein production systems, CombiKARYON™ uses the unique features of the filamentous fungus, *Neurospora crassa*, to create combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology. Strains carrying these new gene sequences are fused to one another in all possible combinations to produce libraries in the following manner.

VARIANT	Light chain 1	Light chain 2	Light chain 3	Light chain 4
Heavy chain 1	L1H1	L2H1	L3H1	L4H1
Heavy chain 2	L1H2	L2H2	L3H2	L4H2
Heavy chain 3	L1H3	L2H3	L3H3	L4H3
Heavy chain 4	L1H4	L2H4	L3H4	L4H4

In this illustration, 16 unique monoclonal antibody combinations are produced from 4 light and 4 heavy chain subunit variants. In a standard microtiter plate configuration, 96 unique combinations would be produced when 12 variants of one subunit are arrayed against 8 variants of the second subunit. With CombiKARYON™, this would be done with 20 total transformations. Traditional protein engineering techniques would require 96 transformations after a complicated reassembly process of the subunit genes. These burdensome steps are eliminated using Neugenesis' combinatorial biology approach. The advantages become more apparent in larger libraries. For example, a 100x100

matrix to create 10,000 combinations would require 200 transformations in the CombiKARYON™ system, and 10,000 transformations using traditional techniques. This technology can also be used to create combinations of more than two subunits, to geometrically increase the diversity. The last step is to screen the combinatorial libraries for new proteins with the desired characteristics.

Applying Combinatorial Biology to Drug Discovery and Improvement

CombiKARYON™ is an expedient approach for companies involved in developing difficult and complex protein therapeutics. The applications of the technology are numerous. For example, Neugenesis' technology may be applied to protein hits to improve the characteristics such as binding capacity or stability. By designing and creating small changes in the original molecule, Neugenesis is able to fine-tune the protein without dramatically changing the protein's core structure, which has already been selected for through years of evolution. This technology can also be applied to protein drug candidates already in pre-clinical and clinical trials. The failure rate of drug candidates in the development process is estimated to be at least 60-80%. Neugenesis' combinatorial biology system may help by providing a means to more efficiently fine-tune these candidates into better, therapeutically useful molecules.

Other potential applications of CombiKARYON™ include drug combination research and hybridization. In drug combination research, combinatorial biology enables rapid and inexpensive creation of any number of combinations of synergistic proteins, which can then be screened for the most effective combination. In hybridization, combinatorial biology can be used to develop hybrid molecules with both binding and effector moieties, improving the specificity of therapeutic agents.

In addition, cultures of desirable molecules identified through this technology can be easily expanded to produce large-scale quantities of the new heteromeric protein for further evaluation, since the protein is already in a *Neurospora* production strain.

Protein Expression | Neugenesis' Homepage | Company Overview | Neugenesis in the News | Technology Tour

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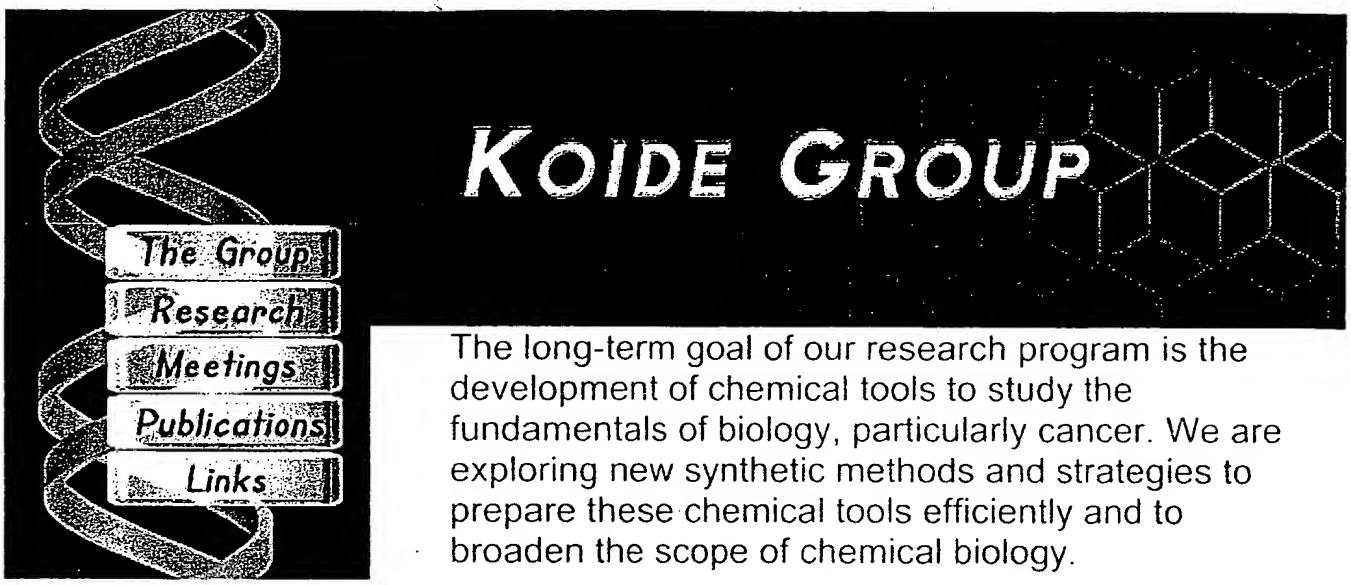

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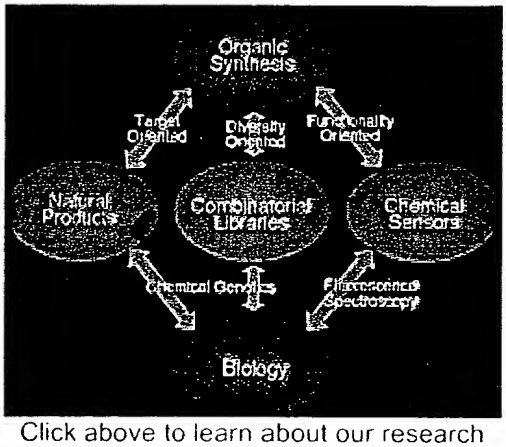

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Glossary of Terms Used in Medicinal Chemistry
(IUPAC Recommendations 1998)

A to H

Contents

Active transport*

Active transport is the carriage of a solute across a biological membrane from low to high concentration that requires the expenditure of (metabolic) energy.

Address-message concept

Address-message concept refers to compounds in which part of the molecule is required for binding (address) and part for the biological action (message).

ADME

Abbreviation for **Absorption, Distribution, Metabolism, Excretion**. (See also **Pharmacokinetics; Drug disposition**).

Affinity

Affinity is the tendency of a mc ule to associate with another. The **affi y** of a **drug** is its ability to bind to its biological target (**receptor**, **enzyme**, transport system, etc.) For pharmacological **receptors** it can be thought of as the frequency with which the **drug**, when brought into the proximity of a **receptor** by diffusion, will reside at a position of minimum free energy within the force field of that **receptor**.

For an **agonist** (or for an **antagonist**) the numerical representation of **affinity** is the reciprocal of the equilibrium dissociation constant of the ligand-**receptor** complex denoted K_A, calculated as the rate constant for offset (k_{-1}) divided by the rate constant for onset (k_1).

Agonist***

An **agonist** is an endogenous substance or a **drug** that can interact with a **receptor** and initiate a physiological or a pharmacological response characteristic of that **receptor** (contraction, relaxation, secretion, **enzyme** activation, etc.).

Allosteric binding sites

Allosteric binding sites are contained in many **enzymes** and **receptors**. As a consequence of the binding to **Allosteric binding sites**, the interaction with the normal ligand may be either enhanced or reduced.

Allosteric enzyme*

An **allosteric enzyme** is an **enzyme** that contains a region to which small, regulatory molecules ("effectors") may bind in addition to and separate from the substrate binding site and thereby affect the catalytic activity.

On binding the effector, the catalytic activity of the **enzyme** towards the substrate may be enhanced, in which case the effector is an activator, or reduced, in which case it is a de-activator or inhibitor.

Allosteric regulation

Allosteric regulation is the regulation of the activity of **allosteric enzymes**. (See also **Allosteric binding sites**; **Allosteric enzymes**).

Analog

An **analog** is a **drug** whose structure is related to that of another **drug** but whose chemical and biological properties may be quite different. (See also **Congener**).

Antagonist***

An **antagonist** is a **drug** or a compound that opposes the physiological effects of another. At the **receptor** level, it is a chemical entity that opposes the **receptor**-associated responses normally induced by another bioactive agent.

Antimetabolite***

An **antimetabolite** is a structural **analog** of an intermediate (substrate or **coenzyme**) in a physiologically

occurring metabolic pathway th lcts by replacing the natural substrate t ; blocking or diverting the biosynthesis of physiologically important substances.

Antisense molecule

An **antisense molecule** is an **oligonucleotide** or **analog** thereof that is complementary to a segment of RNA (ribonucleic acid) or DNA (deoxyribonucleic acid) and that binds to it and inhibits its normal function.

Autacoid

An **autacoid** is a biological substance secreted by various cells whose physiological activity is restricted to the vicinity of its release; it is often referred to as local **hormone**.

Autoreceptor

An **autoreceptor**, present at a nerve ending, is a **receptor** that regulates, via positive or negative feedback processes, the synthesis and/or release of its own physiological ligand. (See also **Heteroreceptor**).

Bioassay***

A **bioassay** is a procedure for determining the concentration, purity, and/or biological activity of a substance (e.g., vitamin, **hormone**, plant growth factor, antibiotic, **enzyme**) by measuring its effect on an organism, tissue, cell, **enzyme** or **receptor** preparation compared to a standard preparation.

Bioisostere

A **bioisostere** is a compound resulting from the exchange of an atom or of a group of atoms with another, broadly similar, atom or group of atoms. The objective of a bioisosteric replacement is to create a new compound with similar biological properties to the parent compound. The bioisosteric replacement may be physicochemically or topologically based. (See also **Isostere**)

Bioprecursor prodrug

A **bioprecursor prodrug** is a **prodrug** that does not imply the linkage to a carrier group, but results from a molecular modification of the active principle itself. This modification generates a new compound, able to be transformed metabolically or chemically, the resulting compound being the active principle.

Biotransformation

Biotransformation is the chemical conversion of substances by living organisms or **enzyme** preparations.

CADD

See **Computer-assisted drug design**.

Carrier-linked prodrug (Carrier prodrug)

A **carrier-linked prodrug** is a **prodrug** that contains a temporary linkage of a given active substance with a transient carrier group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed *in vivo*, usually by a hydrolytic cleavage.

Cascade prodrug

A **cascade prodrug** is a **prodrug** for which the cleavage of the carrier group becomes effective only after unmasking an activating group.

Catabolism***

Catabolism consists of reactions involving endogenous organic substrates to provide chemically available energy (e.g., ATP) and/or to generate metabolic intermediates used in subsequent anabolic reactions.

Catabolite

A **catabolite** is a naturally occurring **metabolite**.

Clone*

A **clone** is a population of genetically identical cells produced from a common ancestor. Sometimes, "**clone**" is also used for a number of recombinant DNA (deoxyribonucleic acid) molecules all carrying the same inserted sequence.

Codon*

A **codon** is the sequence of three consecutive **nucleotides** that occurs in mRNA which directs the incorporation of a specific amino acid into a protein or represents the starting or termination signals of protein synthesis.

Coenzyme

A **coenzyme** is a dissociable, low-molecular weight, non-proteinaceous organic compound (often **nucleotide**) participating in enzymatic reactions as acceptor or donor of chemical groups or electrons.

Combinatorial synthesis

Combinatorial synthesis is a process to prepare large sets of organic compounds by combining sets of building blocks.

Combinatorial library

A **combinatorial library** is a set of compounds prepared by **combinatorial synthesis**.

CoMFA

See **Comparative Molecular Field Analysis**.

Comparative Molecular Field Analysis (CoMFA)**

Comparative molecular field a ysis (CoMFA) is a **3D-QSAR** metho at uses statistical correlation techniques for the analysis of the quantitative relationship between the biological activity of a set of compounds with a specified alignment, and their three-dimensional electronic and steric properties. Other properties such as hydrophobicity and hydrogen bonding can also be incorporated into the analysis. (See also **Three-dimensional Quantitative Structure-Activity Relationship [3D-QSAR]**).

Computational chemistry**

Computational chemistry is a discipline using mathematical methods for the calculation of molecular properties or for the simulation of molecular behaviour.

Computer-assisted drug design (CADD)**

Computer-assisted drug design involves all computer-assisted techniques used to discover, design and optimize biologically active compounds with a putative use as **drugs**.

Congener***

A **congener** is a substance literally *con-* (with) *generated* or synthesized by essentially the same synthetic chemical reactions and the same procedures. **Analogs** are substances that are analogous in some respect to the prototype agent in chemical structure.

Clearly **congeners** may be **analogs** or vice versa but not necessarily. The term **congener**, while most often a synonym for homologue, has become somewhat more diffuse in meaning so that the terms **congener** and **analog** are frequently used interchangeably in the literature.

Cooperativity

Cooperativity is the interaction process by which binding of a ligand to one site on a macromolecule (**enzyme**, **receptor**, etc.) influences binding at a second site, e.g. between the substrate binding sites of an **allosteric enzyme**. Cooperative **enzymes** typically display a sigmoid (S-shaped) plot of the reaction rate against substrate concentration. (See also **Allosteric binding sites**).

3D-QSAR

See **Three-dimensional Quantitative Structure-Activity Relationship**.

De novo* design*

***De novo* design** is the design of bioactive compounds by incremental construction of a ligand model within a model of the **receptor** or **enzyme** active site, the structure of which is known from X-ray or nuclear magnetic resonance (NMR) data.

Disposition

See **Drug disposition**.

Distomer

A **distomer** is the enantiomer o. chiral compound that is the less poten . a particular action. This definition does not excude the possibility of other effect or side effect of the **distomer** (See also **Eutomer**).

Docking studies

Docking studies are molecular modeling studies aiming at finding a proper fit between a ligand and its binding site.

Double-blind study

A ***double-blind study*** is a clinical study of potential and marketed **drugs**, where neither the investigators nor the subjects know which subjects will be treated with the active principle and which ones will receive a placebo.

Double prodrug (or pro-prodrug)

A **double prodrug** is a biologically inactive molecule which is transformed *in vivo* in two steps (enzymatically and/or chemically) to the active species.

Drug***

A **drug** is any substance presented for treating, curing or preventing disease in human beings or in animals. A **drug** may also be used for making a medical diagnosis or for restoring, correcting, or modifying physiological functions (e.g., the contraceptive pill).

Drug disposition

Drug disposition refers to all processes involved in the absorption, distribution **metabolism** and excretion of **drugs** in a living organism.

Drug latentiation

Drug latentiation is the chemical modification of a biologically active compound to form a new compound, which *in vivo* will liberate the parent compound. **Drug latentiation** is synonymous with **prodrug** design.

Drug targeting

Drug targeting is a strategy aiming at the delivery of a compound to a particular tissue of the body.

Dual action drug

A **dual action drug** is a compound which combines two desired different pharmacological actions at a similarly efficacious dose.

Efficacy

Efficacy describes the relative intensity with which **agonists** vary in the response they produce even

when they occupy the same nu. .r of **receptors** and with the same **affi .** . **Efficacy** *is not* synonymous to **Intrinsic activity**.

Efficacy is the property that enables **drugs** to produce responses. It is convenient to differentiate the properties of **drugs** into two groups, those which cause them to associate with the **receptors** (**affinity**) and those that produce stimulus (**Efficacy**). This term is often used to characterize the level of maximal responses induced by **agonists**. In fact, not all **agonists** of a **receptor** are capable of inducing identical levels of maximal responses. Maximal response depends on the efficiency of **receptor** coupling, i.e., from the cascade of events, which, from the binding of the **drug** to the **receptor**, leads to the observed biological effect.

Elimination

Elimination is the process achieving the reduction of of the concentration of a **xenobiotic** including its **metabolism**.

Enzyme*

An **enzyme** is a macromolecule, usually a protein, that functions as a (bio) catalyst by increasing the reaction rate.

In general, an **enzyme** catalyzes only one reaction type (reaction selectivity) and operates on only one type of substrate (substrate selectivity). Substrate molecules are transformed at the same site (regioselectivity) and only one or preferentially one of chiral a substrate or of a racemate is transformed (enantioselectivity[special form of stereoselectivity]).

Enzyme induction*

Enzyme induction is the process whereby an (inducible) **enzyme** is synthesized in response to a specific inducer molecule. The inducer molecule (often a substrate that needs the catalytic activity of the inducible **enzyme** for its **metabolism**) combines with a repressor and thereby prevents the blocking of an operator by the repressor leading to the translation of the gene for the **enzyme**.

Enzyme repression*

Enzyme repression is the mode by which the synthesis of an **enzyme** is prevented by repressor molecules.

In many cases, the end product of a synthesis chain (e.g., an amino acid) acts as a feed-back corepressor by combining with an intracellular aporepressor protein, so that this complex is able to block the function of an operator. As a result, the whole operation is prevented from being transcribed into mRNA, and the expression of all **enzymes** necessary for the synthesis of the end product **enzyme** is abolished.

Eudismic ratio

Eudismic ratio is the **potency** of the **eutomer** relative to that of the **distomer**.

Eutomer

The **Eutomer** is the enantiomer a chiral compound that is the more po for a particular action (See also **Distomer**).

Genome*

A **genome** is the complete set of chromosomal and extrachromosomal genes of an organism, a cell, an organelle or a virus; the complete DNA (deoxyribonucleic acid) component of an organism.

Hansch analysis**

Hansch analysis is the investigation of the quantitative relationship between the biological activity of a series of compounds and their physicochemical substituent or global parameters representing hydrophobic, electronic, steric and other effects using multiple regression correlation methodology.

Hapten***

A **hapten** is a low molecular weight molecule that contains an antigenic determinant but which is not itself antigenic unless combined with an antigenic carrier.

Hard drug

A **hard drug** is a nonmetabolizable compound, characterized either by high lipid solubility and accumulation in adipose tissues and organelles, or by high water solubility.

In the lay press the term "**Hard Drug**" refers to a powerful **drug** of abuse such as cocaine or heroin.

Heteroreceptor

A **heteroreceptor** is a **receptor** regulating the synthesis and/or the release of mediators other than its own ligand (See also **Autoreceptor**).

Homologue

The term **homologue** is used to describe a compound belonging to a series of compounds differing from each other by a repeating unit, such as a methylene group, a peptide residue, etc.

Hormone***

A **hormone** is a substance produced by endocrine glands, released in very low concentration into the bloodstream, and which exerts regulatory effects on specific organs or tissues distant from the site of secretion.

Hydrophilicity**

Hydrophilicity is the tendency of a molecule to be solvated by water.

Hydrophobicity**

Hydrophobicity is the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non polar molecules. (See also **Lipophilicity**).

Continue with terms starting with I to X.

Return to home page for **Glossary** of Terms Used in Medicinal Chemistry.